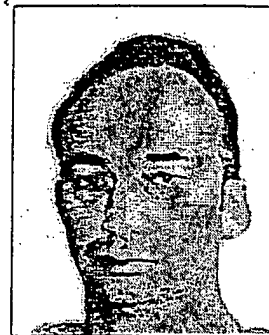


Molecular Basis for Designing Selective Modulators of Retinoic Acid Receptor Transcriptional Activities

Philippe Lefebvre*

INSERM U 459, Faculté de Médecine Henri Warembourg, 1, place de Verdun, 59045 Lille cedex, FRANCE



Abstract: Retinoic acid receptors are ligand-regulated transcription factors belonging to the nuclear receptor superfamily, which comprises 49 members in the human genome. *all-trans* retinoic acid and 9-*cis* retinoic acid receptors (RARs and RXRs) are each encoded by three distinct genes and several isoforms arise from alternative splicing and the use of different promoters. While RXRs are promiscuous dimerization partners of several other nuclear receptors, RARs are active, *in-vivo*, when associated to RXRs. Retinoids are therefore regulators of multiple physiological processes, from embryogenesis to metabolism. Different combinations of RXR:RAR heterodimers occur as a function of their tissue-specific expression and their activity is mostly conditioned by the activation status of RAR. These heterodimers are defined as non permissive heterodimers, in opposition to permissive dimers whose transcriptional activity may be modulated through RXR and its dimerization partner. The transcriptional activity of these dimers also relies on their ability to recruit nuclear coactivators and corepressors, which function as multi proteic complexes harboring several enzymatic activities (acetylases, kinases). The structure of the ligand bound to the RAR moiety of the dimer, as well as the nature of the DNA sequence to which dimers are bound, dictate the relative affinity of dimers for coactivators and thus its overall transcriptional activity. RARs are also able to repress the activity of unrelated transcription factors such as AP1 and NF- κ -B, and therefore have potent anti proliferative and anti inflammatory properties. This review summarizes our current view of molecular mechanisms governing these various activities and emphasizes the need for a detailed understanding of how retinoids may dictate transactivating and transrepressive properties of RARs and RXRs, which may be considered as highly valuable therapeutic targets in many diseases such as cancer, skin hyperproliferation and metabolic disorders (diabetes, atherosclerosis etc).

INTRODUCTION

The concept of nuclear receptors emerged from seminal observations made by E.V. Jensen and collaborators in the early 60's, demonstrating the specific binding of radioactive estradiol by target organs for this hormone, and therefore hinted at a molecular relay mediating the biological effects of this steroid. In the mid-60's, the conceptual framework for modern endocrinology was set, introducing notions of transcriptional regulation [2, 3] and chromatin remodeling upon steroid treatment of target cells. Further investigations by G.M. Tomkins and collaborators, using the tyrosine amino transferase gene as a model system, led to the proposal of a direct transcriptional regulation of genes by steroids. The concept of receptor activation, as defined by the acquisition of a DNA binding activity by the receptor, was proposed a few years later [6,7].

Further efforts aimed at the purification of steroid receptors, allowing to suspect the oligomeric nature of steroid receptors [8] and to show the existence of structural

domains within the glucocorticoid receptor [9]. Beside the intense body of work devoted to the characterization of the hetero oligomeric nature of steroid receptors (reviewed in [10]), these proteins have been defined as ligand-regulated transcription factors on the basis of their specific binding to short DNA sequences or hormone responsive elements (HRE) in the promoter of hormonally responsive genes [11-16]. Chromatin structure alteration of a steroid-regulated promoter was shown to be concomitant to hormone treatment of target cells [17]. A major breakthrough was the cloning of several steroid receptors (reviewed in [18]), which paved the road for a detailed molecular study of the ever growing superfamily of nuclear receptors. After more than two decades of intense research in this field, nuclear receptors appear as major players in embryonic development, cell differentiation and proliferation processes and as regulators of cellular homeostasis. Not surprisingly, these functions also identified them as very promising therapeutic targets in a number of pathological states such as cancer, inflammation, and metabolic disorders.

Among the members of this superfamily, all *trans* retinoic acid (atRA) receptors (RARs) and 9-*cis* retinoic acid receptors (RXRs) became the focus of intense scrutiny since they are the molecular relays not only of pleiotropic effects of retinoic acids (atRA, 9-*cis* RA, 4oxo RA and 3,4 dihydroRA) on cell growth, differentiation and apoptosis,

* Address correspondence to this author at the INSERM U 459, Faculté de Médecine Henri Warembourg, 1, place de Verdun, 59045 Lille cedex, FRANCE; Tel 33-3-20-62-68-87; Fax 33-3-20-62-68-84; email: p.lefebvre@lille.inserm.fr

but also of potent anti proliferative and anti inflammatory effects of natural and synthetic retinoids. Although their many potential therapeutic applications are limited due to a high number of side effects, retinoids proved to be useful in dermatological diseases (acne, psoriasis, photoaging) precancerous lesions, and cancers [19]. RARs are encoded by three different genes (isotypes α , β and γ) and occurs as multiple isoforms in mouse (α 1, α 2, β 1 to β 4, and γ 1 and γ 2), much like RXRs (isotypes α , β and γ and isoforms α 1 to 3, β 1 and 2, γ 1 and 2). Importantly, RXRs are heterodimeric partners not only for RARs, but also for other nuclear receptors, including thyroid hormone receptors (TRs), vitamin D receptor (VDR), peroxisome proliferator activated receptors (PPARs) and a number of orphan nuclear receptors, making of retinoids signaling molecules at the crossroad of multiple signaling pathways. Therefore the design of functionally selective retinoids is a challenge to the molecular pharmacologists, and I will in this review describe the molecular mechanisms of retinoid action which may serve as a basis for designing pharmacological agents allowing the selective modulation of retinoid-sensitive genes.

phosphorylation of key amino acids. The DNA binding domain (DBD) or domain C, is highly conserved between members of this superfamily and contains two C4-type zinc fingers. The N-terminal zinc finger is directly involved in the recognition of specific DNA response elements, while the C-terminal finger serves as a binding interface for dimerization partners and stabilizes the globular structure of the DBD. The domain D, or hinge region, contains amino acids that in some cases stabilize receptor-DNA interactions and a lysine-rich nuclear localization signal. The large C-terminal region (domain E or LBD), which accounts for almost half of the protein, is a multi functional domain involved in dimerization, ligand binding and nuclear coactivator (NCoAs) and corepressor (NCoRs) recruitment. It contains the activation function2 (AF2) which is ligand-dependent and directly involved in NCoAs and NCoRs binding to the receptor. In some cases, a small C terminal domain (domain F) occurs, the function of which being poorly characterized. However, we demonstrated that the F domain of RAR α modulates the transcriptional activity of this receptor [20]. Such a modulatory role of the F domain has also been documented for the estrogen receptor [21].

STRUCTURE OF NUCLEAR RECEPTORS

Sequence analysis of nuclear receptor cDNAs allowed the identification of highly conserved domains. With the help of biochemical and functional data, functions were ascribed to these homologous regions which are depicted in Fig. (1). Nuclear receptors are therefore, as all other transcription factors, formed of several functionally autonomous domains, which however interact with each other to modulate receptor functions. The amino terminal domain is highly variable, even absent in some receptors such as VDR, and contains the activation function 1 (AF1). Transcriptional activation by AF1 is ligand-independent and cell-specific, and the activity of AF1 is mostly affected through direct

RETINOIC ACID RECEPTORS

The structural and functional organization of RARs and RXRs is thus analogous to that of other steroid and nuclear receptors. RARs and RXRs are constitutively nuclear and while most of experimental data obtained *in-vitro* show that these receptors bind DNA as heterodimers in the absence of ligand, some arguments based on *in-vivo* footprinting [22,23] experiments and on the role of antagonists in regulating the receptor dimerization process [24] suggest that ligand binding has a critical role in at least increasing the affinity of RXR:RAR heterodimers for DNA in intact cells. DNA sequences recognized by RXR:RAR dimers are generally direct repeats of the AGGTCA motif. The spacing

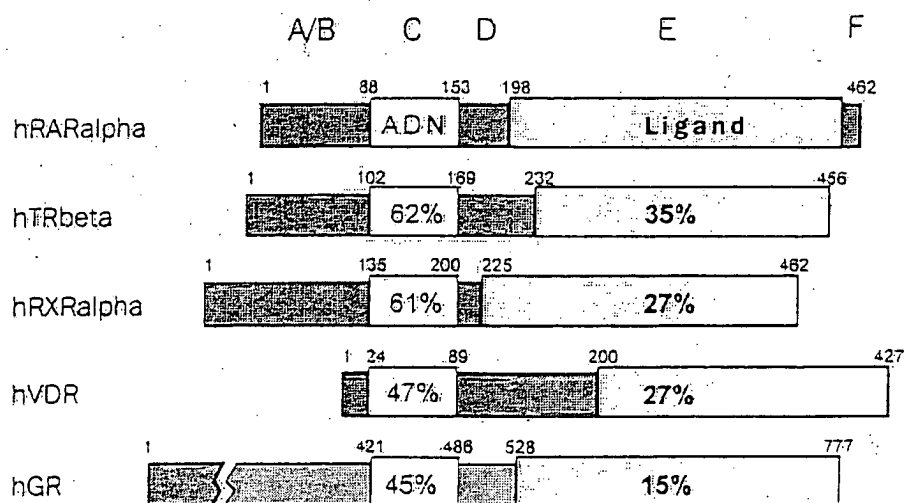


Fig. (1). Structural organization of nuclear receptors. The structural and functional organization of some representative members of the nuclear receptors family is depicted here. Numbers indicate amino acids position along the sequence and percentages indicate the percentage of amino acid homology between each domain with RAR α as the reference receptor.

of the two half sites of the response element dictates its specificity for a given RXR-containing dimer, with RXR:RAR dimers binding preferentially to DR1, DR2 and DR5 retinoic acid response elements (RARE). Spacing of the two half sites also imposes the use of different dimerization interfaces located in the DBD of each receptor [25,26]. This led to the proposal of the "1-2-3-4-5 rule" [27], which is however far from being stringent and is notably dependent on the promoter context [28]. RXR:RAR heterodimers are classified as non permissive heterodimers, indicating the fact that the 5' bound receptor is transcriptionally silenced, yet fully competent for ligand binding, and that this transcriptional silencing is relieved only when the 3' bound

receptor is liganded. The importance of the receptor binding polarity is exemplified by the inability of RXR-specific ligands to activate RXR:RAR heterodimers bound to DR5 or DR2 response elements, and that the RXR-RAR transcriptional synergy is observed only when RAR is liganded [29,30].

The structure of non liganded (apo) RXR and RAR LBDs, as well as that of liganded (holo) RXR and RARs LBDs have been solved [31-34], and recently the structure of RXR:RAR LBDs heterodimers has been described [35]. The structural organization of this multi functional domain is conserved throughout the nuclear receptor superfamily, and is

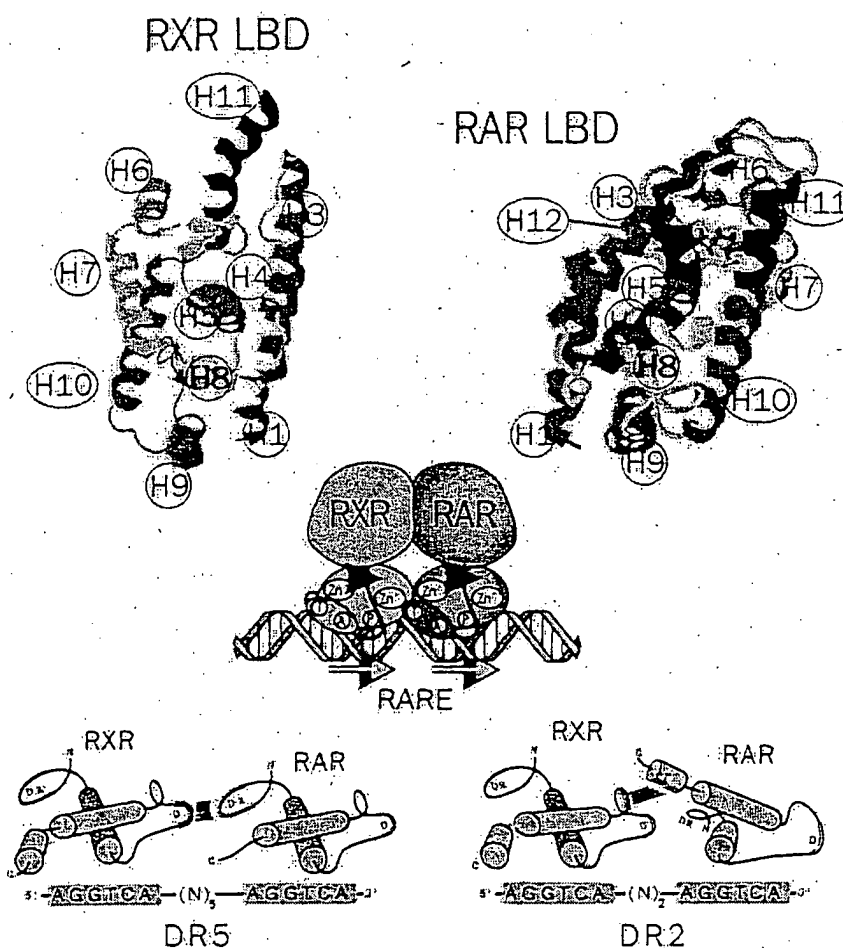


Fig. (2). Salient structural features of RXR:RAR heterodimers. The general spatial organization of RXR:RAR heterodimers is shown at the center of the figure, with RXR being the 5'-bound receptor. The three-dimensional structure of apoRXR and holoRAR is shown at the top, evidencing structural transitions in the RAR molecule which are detailed in the text and in Figure 3. At the bottom is depicted the alternative use of dimerization interfaces in the DBD upon binding to DR5 or DR5 RAREs. Data were compiled essentially from references [25, 26, 27, 29-32].

characterized by a common fold made of 12 α -helices and a β -turn arranged as a three layered anti parallel α -helices scaffold [see Fig. (2)]. The ligand-receptor interaction is mediated through amino acids side chains located in helices H1, 3, 5 and 12 [32] can be obtained through specific interactions of the ligand with amino acids unique to each isotype [32, 36-38].

Agonist binding triggers structural transconformations which were first evidenced by altered resistance to protease digestion (see [39] and references therein) and to date, three conformational states of retinoic acid receptor LBDs have been crystallized: apo-LBD, agonist-bound LBD and antagonist bound-LBD. The transition from a transcriptionally inactive, unliganded form of the receptor to a transcriptionally active, agonist-bound receptor is characterized by a more compact overall structure and a strikingly different position of helix 12 [Fig. (3)], previously identified as required for the transcriptional activity of nuclear receptors [40]. This helix, largely exposed to the solvent in the apo receptor, become tightly folded along the LBD core structure, establishing salt bridges with amino acids located in helix 3 and/or 4 which are essential to, but differentially involved in transcriptional activation by natural and synthetic retinoids [41]. AF-2 positioning is modified in the antagonist-bound structure, supporting the view that ligand structure directly impinges on receptor conformation and allows, or not, the formation of an interface suitable for coactivator recruitment. This view is further strengthened by mutagenesis experiments showing that alteration of the

structure of the H11-H12 loop has an impact on RAR transcriptional activity as a function of ligand structure [20,42]. Finally, other structural changes affect helices 3, 6 and 11, yielding a polypeptide with inactivated interaction surfaces for corepressors binding and new functional interfaces suitable for coactivator recruitment.

While agonist binding strongly alters these protein-protein interaction interfaces, it is surprising to note that it has no noticeable effect on the dimerization interface as determined by crystallographic studies. This interface consists mostly of helices 9 and 10 [35] in which some specific amino acids appear to be more specifically involved in homo- or heterodimerization with RXR [43]. However, we have shown that RAR antagonists are characterized by their ability to inhibit RAR:RXR dimerization in solution, both *in-vitro* and *in-vivo* [24]. These data and others indicated that within a dynamic context, ligand structure perturbs the structural integrity of dimerization interfaces or of neighboring sequences. DNA-independent dimerization is dependent on the ability of helix 12 to establish salt bridges with amino acids from helices 3 and 4 [24].

TRANSCRIPTIONAL ACTIVATION BY RARs AND RXRs

When expressed at high levels, steroid receptors interfere with each other's ability to induce transcription from regulated promoters [44]. This observation provided the first

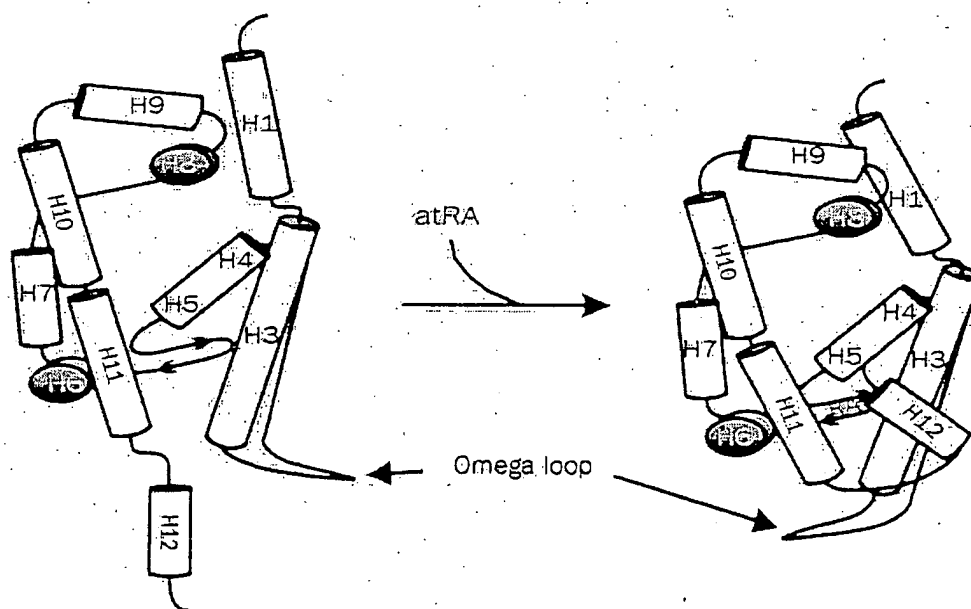


Fig. (3). Structural transitions in the LBD of RARs. The monomeric, apo-LBD is characterized by a relaxed conformation in which the AF2 (helix 12) is accessible to the solvent. Ligand binding induces major structural alterations which are detailed in the text. Flipping of the omega loop and repositioning of H12 are particularly evident in the holo-RAR structure, as well as expelling of helix 11.

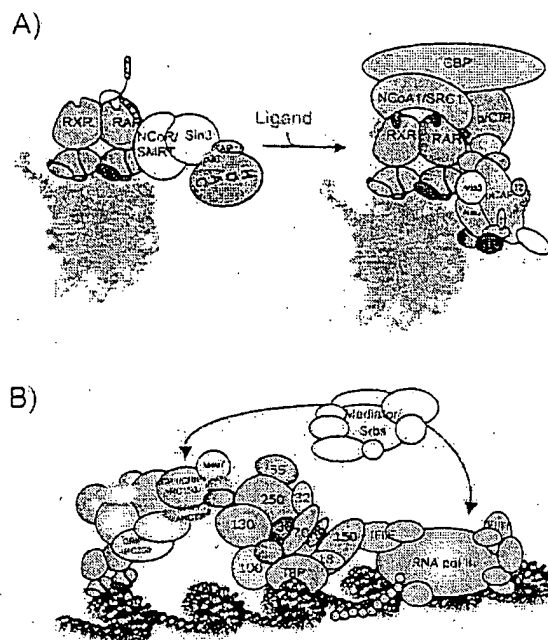


Fig. (4). Transcriptional activation by RARs: Two mechanisms are proposed. A) Transactivation through recruitment of p160 coactivators and of CBP. The RXR:RAR dimer is shown as constitutively bound to nucleosomal DNA, also evidence exist showing that ligand promotes dimerization and DNA binding *in-vivo* (see text). Agonist binding induces NCoA dissociation, and NCoA recruitment. Additionally, pCAF interacts with domain D of RAR. B) An analogous model is proposed for the recruitment of the mediator complex to RXR:RAR dimers.

evidence for the existence of common factors required for nuclear receptor-induced transcription, and expressed in limiting amounts in target cells. These proteins are defined as transcriptional coactivators based on their ability to interact with liganded receptors and to increase their transcriptional activity, since NCoAs are functional only upon recruitment by nuclear receptors. Several experimental strategies have been used to successfully identify potential coactivator proteins, including the yeast two-hybrid system [45-47] and affinity chromatography [48]. Several types of transcriptional coactivators have been isolated and include factors of the p160 family, the DRIP/ARC/TRAP/mediator complex, CBP/p300 and other intermediary factors [Fig. (4)].

The SRC (steroid receptor coactivator) family is the most prominent set of proteins that has been characterized so far and includes SRC1, TIF2/GRIP1 and ACTR/pCIP/RAC3/AIB1. SRC1 was the first coactivator to be cloned [49] and has been extensively characterized. It interacts with many nuclear receptors, including RAR and RXR, and may mediate NF- κ B transcriptional enhancement (reviewed in [50]). While not displaying strong overall sequence homology, the p160 coactivators are characterized by a common structural organization in which a bHLH-PAS is found at the N-terminus of the protein. It is of interest to note that SRC1 and ACTR display moderate acetylase activities which may be involved in histone acetylation [51,52] and promoter desensitization [53]. The bHLH domain is usually involved in DNA binding in other transcription factors, but has no known function in p160 coactivators. Similarly, the PAS domain, found in transcription factors such as *period*, the aryl hydrocarbon

(dioxin) receptor and *single-minded*, generally plays a role in protein-protein interactions which is not yet documented for p160 factors. SRC1 contains an S/T rich region and two Q-rich regions. The central portion of this protein bears a functionally critical domain which is the nuclear receptor interaction domain (RID), and contains three LXXLL motifs. It spans 140 amino acids and each LXXLL motif is separated by about 50 amino acids. The LXXLL motif is absolutely required for nuclear receptor:coactivator interaction and is present in TIF1, p160 coactivators, DRIP205/TRAP220 (reviewed in [54] and [55]) and other coactivators such as CIA. Finally, a CBP/p300 interaction domain is also found in the central region of SRC1, which contains three LXXLL motifs involved in CBP:p300 binding. This domain is an autonomous transcription activation domain whose function is dependent on the interaction with CBP/p300, [57,58].

CBP/p300 are transcriptional cofactors for a number of transcription factors, including CREB and AP-1 [59]. p300 was shown to associate to estrogen receptor α [60] and to other nuclear receptors including RARs [61-63]. Several lines of evidence suggest that CBP/p300 associates strongly to nuclear receptors through an interaction with p160 coactivators [64]. Both proteins displays a histone acetyltransferase (HAT) activity [65], however, inactivation of HAT activity of CBP or SRC1 does not affect RAR-mediated transactivation [66]. Since CBP/p300 can potentially associate to several transcription factors, it has been assumed that it may serve as a cointegrator for multiple signaling pathways [67].

Large, megadalton-sized complexes interacting with nuclear receptors (TRAP [68,69] and DRIP [48, 70]) and

other transcription factors such as SREBP-1a (ARC, [71,72]), and E1A [73]), or with human SRB subunits (NAT [74] and SMCC [75]) have been isolated from human cells nuclear extracts. These complexes contain both novel proteins and polypeptides homologous to components of yeast Mediator and are devoid of HAT activity [70]. However, the DRIP complex is a potent coactivator in a chromatin context, suggesting that this complex may associate with HATs, as shown in yeast [76].

Other coactivators for RARs have been described in the literature (for recent and extensive reviews, please refer to [50,55], and [77]). pCIP, a protein interacting with CBP, is highly related to SRC1 and is required together with CBP for efficient RAR-mediated transcription. pCAF also interacts with CBP/p300 and its HAT activity is necessary for RAR transcriptional activity [66,78]. CARM-1 (coactivator associated arginine methyl transferase 1) has been identified on the basis of its interaction with a p160 coactivator, GRIP1. This protein contains a strong histone methyl transferase activity selective for histone H3 [79,80].

Thus RARs and other nuclear receptors associate in a ligand-dependent manner with multi protein complexes possessing multiple enzymatic activities catalyzing post translational modifications such as phosphorylation (TIF1, [81], acetylation (SRC1, CBP/p300, pCAF) and methylation (CARM-1). Each of these component may control either chromatin compaction or coactivator complexes stability. A highly debated question is how these different complexes stimulate NR-dependent transcription, and whether they may provide specific routes for transcriptional activity for a given receptor. In keeping with this, some structural arguments have been put forward to suggest that a level of specificity of interaction between receptors and coactivators may be achieved.

As mentioned above, RIDs from NCoAs contain conserved LXXLL motifs essential for their ligand-dependent interaction with receptors. Crystallographic data show that these RIDs form amphipathic helices which interact with the hydrophobic groove created upon folding of helix 12 and repositioning of helices 3, 4, and 5 in the holo receptor structure [35,82,83]. Mutational analysis of the three RIDs from SRC1 and TIF2 showed that none of these motifs is individually required for interaction [84]. A more refined analysis showed however that the central (NR2) motif is the preferred motif for interaction with RAR, and also RXR and ER. The specific requirement for nuclear receptor binding to NCoAs through NR1, NR2 and NR3 motifs allowed the definition of a receptor code. It is therefore likely that structural features of the receptor as well as the two and three dimensional organisation of NR motifs play a critical role in this recognition code [58,85-87]. It is also worth noting that amino acids flanking the LXXLL motif contribute to binding selectivity of NCoAs to receptors [58,87,88].

TRANSCRIPTIONAL INTERFERENCE BY RARs AND RXRs

Transrepression is the second activity of NRs which is characterized by their ability to repress transcription factors

such as AP1 and NF-kappa B in a variety of cell lines. A number of ligands for nuclear receptors, including glucocorticoids (glucocorticoid receptor, GR), retinoids (retinoic acid receptor RARs and RXRs) and fatty acids (peroxisome proliferator activated receptors, PPARs) display such repressive activity, which seems to be the basis for their beneficial therapeutic effects as anti inflammatory and/or anti proliferative agents.

AP1 response elements (TGAC/GTCA) are cis-acting DNA sequences mediating transcriptional responses to many physiological and pharmacological stimuli, including phorbol esters, growth factors, mitogenic hormones, UV and other cellular stresses. These DNA sequences were originally defined as TPA (12-O-tetradecanoylphorbol-13-acetate) response elements which bind dimers of proteins belonging to the bZIP transcription factor family [89] and are found in the promoter of genes involved in cell growth, cell differentiation or cytokine production. These transcription factors include products of immediate-early genes such as members of the fos family (c-fos, FosB, Fra1 and Fra2) and of the jun family (c-jun, junB and junD) [90]. Jun family members may homodimerize on AP1 binding sites, but jun-fos heterodimers have higher affinity for DNA and are more stable, leading to preferential formation of jun:fos heterodimers *in-vivo*.

The transcriptional activity of the AP1 complex is regulated through different modes. First, its activity is determined by the composition of the AP1 dimer which may, according to its molecular composition, display different affinity for a given response element [91]. Therefore, the level of expression of dimerization partners is one key step of AP1 regulation through modulation of the number of possible combinations of dimers. Numerous stimuli such as cAMP, calcium, mitogen-activated protein kinases (MAPK) activators (growth factors, cytokines etc) and JAK tyrosine kinases activators (interferons, EPO, interleukins etc) up regulate c-fos expression and thus favor the formation of c-fos containing dimers. c-jun expression level is regulated through an ATF2/c-jun response element located in the promoter of its gene, which confers responsiveness to a wide range of stimuli known to activate the jun N-terminal kinase (JNK), a member of the MAPK family [92]. Second, post translational modifications exert a strong control on AP1 activity, mostly through regulation of the phosphorylation state of AP1 components. Notably, phosphorylation of c-jun at Ser63 and Ser73 increases its affinity for the coactivator CBP without altering its DNA binding activity [93]. This post translational regulation is controlled through activation of the SAPK signaling module, leading to the activation of JNK. c-fos is exclusively activated by the fos regulating kinase (FRK) through a TPA-insensitive, ras-dependent mechanism [94]. Thus, AP1 activity is regulated through a complex network of protein kinases which renders this transcription factor highly reactive to extracellular stimuli.

The AP1 complex regulates the expression of several genes involved in oncogenic transformation and cellular proliferation such as metalloproteases, VEGF and TGF- β . Moreover, AP1 transactivation is required for tumor promotion *in-vivo* [95]. There is therefore a considerable interest in identifying compounds able to down-regulate AP1

activity and thereby to oppose unregulated cell growth leading to benign or malignant hyperplasia and cancer. Several explanations have been put forward to provide a model for retinoid interference with membrane receptor-controlled signaling pathways. c-jun homodimers binding to AP1 binding sites was shown to be inhibited by RAR α *in-vitro* [96]. Competition for a limiting amount of the coactivator CBP has also been suggested as a relevant mechanism for transrepression of AP1 by retinoids [62]. Interference may also occur through regulation of the enzymatic activity of protein kinases. JNK has been in this respect demonstrated to be a target for retinoid action [97], and this effect is dependent on the transactivating potential of RAR [98]. The description of dissociated retinoids, which are unable to elicit a transactivating response by RARs but yet induce AP1 transrepression [99,100] as well as receptor mutants with altered transactivating capacity but with wild type transrepressive properties [20], clearly suggest that molecular determinants governing the transrepressive activity of RAR are distinct from those ruling its transactivating potential.

PHARMACOLOGICAL MODULATION OF RAR ACTIVITY. THERAPEUTIC APPLICATIONS OF RETINOIDS AND ASSOCIATED SIDE EFFECTS

The interest of retinoids in treatment of skin disorders (acnea, psoriasis, photoaging) and in cancer chemotherapy has been first recognized upon the successful use of atRA, 13-cis RA and etretinate. Moreover, consumption of vitamin A or of its precursor β -caroten is associated to cancer chemoprevention [101]. Retinoid effects on cellular differentiation and/or apoptosis, alone or in association with other differentiating agents such as cytokines, growth factors are increasingly characterized and therapeutic usefulness of these associations have been and are still currently tested (reviewed in [101,102]). One of the most striking effect of retinoids is their ability to promote cellular differentiation of acute promyelocytic leukemia cells in humans [103]. Other positive effects have been reported in myelodysplasia and skin T-lymphoma, however these results remain controversial. Retinoids are also used in chemoprevention of lung cancer and have been tested alone or in combination with interferon in cervix cancer [104].

Curative properties of retinoids in skin disorders have been used for the treatment of acne, psoriasis and hyperkeratinization. Retinoids inhibit Kaposi sarcoma cell proliferation and this effect is associated to positive clinical effects [105-107].

Unfortunately, these beneficial effects are associated to severe side effects such as developmental abnormalities in embryo, hypertriglyceridemia, general mucocutaneous toxicity, headache etc. Moreover, prolonged atRA treatment is associated to multiple side effects such as bone pain, retinoic acid syndrome, renal failure, thrombosis and metabolic resistance [108]. The design of receptor isotype-selective, as well as of function specific retinoids is required to achieve an optimized therapeutical index of retinoids [Fig. (5)]. One of the rationale for designing selective retinoids is

the tissue-specific expression of RAR isotypes. For example, RAR γ is predominantly expressed in skin and the use of RAR γ -selective retinoids is associated to decreased skin irritation [109].

atRA is also used in differentiation therapy of acute promyelocytic leukemia [110] but induces cases of atRA resistance. The chromosomal translocation t(15;17) which is specifically associated with APL, fuses the PML gene to the RAR α locus. Here again, the use of RAR α -selective retinoids may prove to be useful for achieving a better therapeutical index, and the successful use of the RAR α -selective retinoid Am80 in atRA-resistant AML demonstrates that such a strategy is effective [111, 112].

Many genes associated to hyperproliferation, extracellular matrix integrity and inflammation are controlled, at least in part, through AP1 enhancer sequences. Retinoids, as mentioned above, are able to inhibit AP1 activity through interference with MAPK signaling modules. This activity is clearly the basis for carcinogenesis inhibition in mice by retinoids [113], therefore strengthening the anti inflammatory and anti proliferative properties of retinoids by synthesizing transcriptionally inactive retinoids is likely to extend the use of retinoids to treatments of various forms of cancer other than leukemia. Vascular Endothelial Growth Factor (VEGF) expression is increased in hyperproliferative diseases, and controlled in part through AP1 response elements. VEGF expression was inhibited *in-vivo* by a retinoid displaying only transrepressive activity [114], suggesting the general usefulness of dissociated retinoids in the treatment of metastatic cancers [115] through their anti angiogenic properties [116]. Note however that AP1 antagonism is not required in some tissues for observing anti proliferative effects of retinoids [117], underlining the well known tissue-specific effect of retinoids on tumor formation [118].

CONCLUSIONS AND PERSPECTIVES

Retinoids are key regulators of differentiation, proliferation and inflammation. Their successful use in the treatment of skin diseases and neoplasias underlines the need for developing selective modulators of RARs functions, not mentioning the very important role of RXR-specific ligands as modulators of other signaling pathways such as PPAR, extending the possible therapeutical repertoire of these molecules to metabolic diseases such as type II diabetes and atherosclerosis. The concept of selective modulators has been initially proposed for estrogen receptors, for which some ligands (tamoxifen) elicit cell-selective agonist or antagonist activity (reviewed in [119]). The potential importance of retinoid structure in specifying RAR transcriptional or transrepressive activity was strongly emphasized by the synthesis of many retinoids, allowing researchers to decipher the molecular basis of retinoid action. The molecular pharmacology of retinoids suggests that at least four types of activities may result from ligand binding to the receptor: (i) antagonist, (ii) transrepressive, (iii) agonist, (iv) inactive. One major determinant of the agonist activity is the ability of retinoids to promote NCoA recruitment to the promoter. Synthetic retinoids displaying similar affinities for the

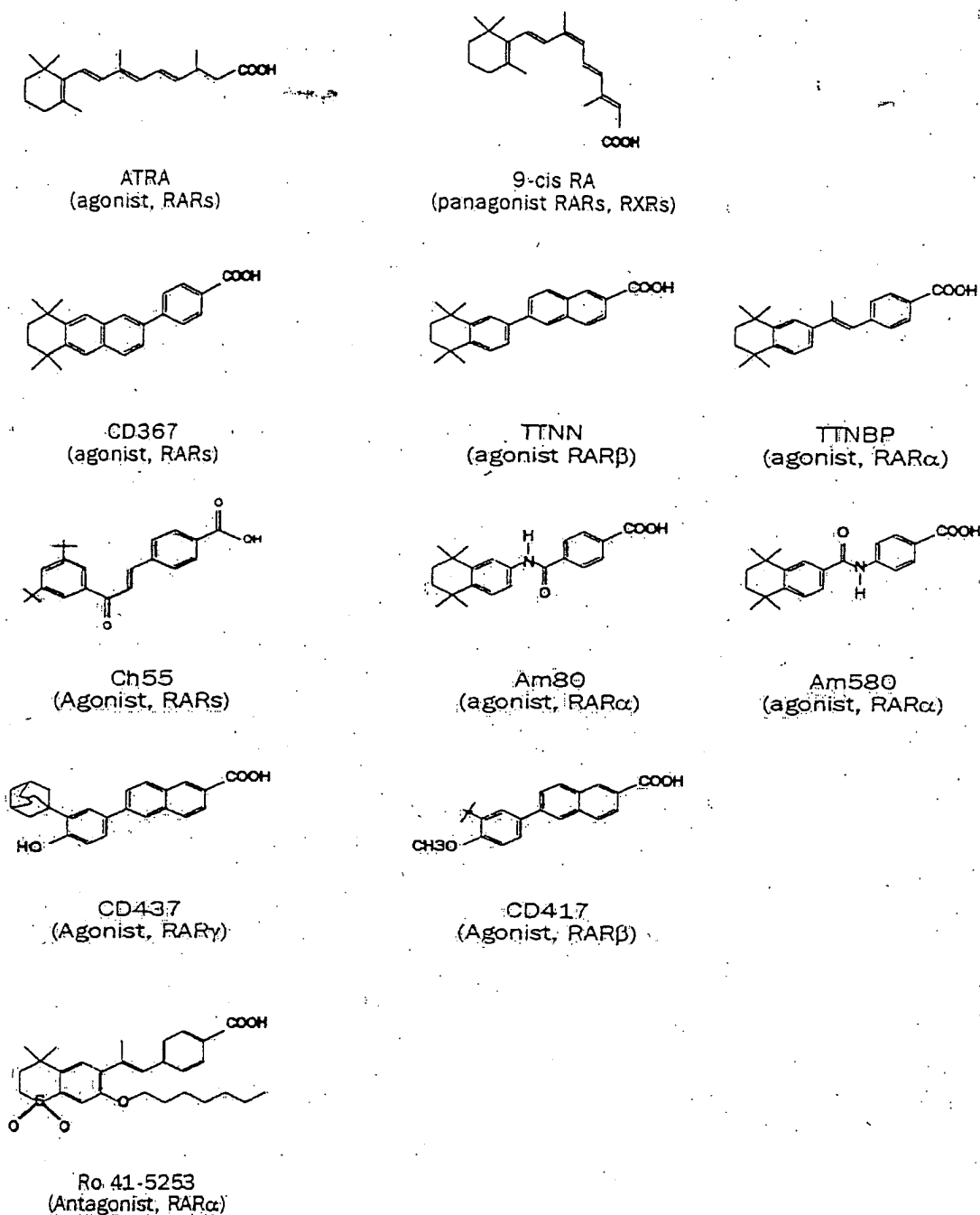


Fig. (5). Chemical structures of several natural and synthetic retinoids. The selectivity and the biological activity of each molecule is indicated between brackets.

monomeric RAR α show dramatically different abilities to activate reporter genes in HeLa cells [49] and endogenous genes in P19 cells (unpublished observations). RAR-selective ligands have distinct quantitative activation properties which are reflected by their ability to promote interaction of DNA-bound hRXR α /hRAR α heterodimers with the nuclear receptor co-activator (NCoA) SRC-1 *in*

vitro. The hormone response element core motifs spacing defined the relative affinity of liganded heterodimers for two NCoAs, SRC-1 and RIP140. hRXR α AF2 was critical to confer hRAR α full responsiveness, but not differential sensitivity of hRAR α to natural or synthetic retinoids. Thus ligand structure instigates a given receptor conformation, and therefore activity. This property was further documented by

the use of RAR α mutants, whose transcriptional activities were distinct and were a function of the ligand structure [20,42]. Furthermore, DNA and RXR also exert allosteric regulations on hRAR α conformation organized as a DNA-bound heterodimer. Thus imposing physically distinct NCoA binding interfaces to the receptor may be important in controlling specific genes by conformationally restricted ligands, and may restrict the pleiotropic effects of natural retinoic acids. Ligand structure also appeared to alter dimerization interfaces, since RAR antagonists display a distinctive feature which is their ability to prevent RXR:RAR heterodimers formation *in-vivo* and *in-vitro*. Thus some degree of gene-selective induction or repression may be achieved in a tissue-specific manner when using appropriately designed compounds. Therefore the elucidation of molecular mechanisms governing the activities of RARs and RXRs, as well as the identification of other signaling pathways which may alter receptor function by post translational modifications [120], will pave the way for the synthesis of new pharmacological tools with potential applications in multiple pathological states.

ACKNOWLEDGEMENTS

The author wishes to thank current and past members of the "Molecular Biology of Nuclear Receptors" group, and Dr C. Rachez for critical review of the manuscript. This work was supported by grants from Institut National de la Santé et de la Recherche médicale (I.N.S.E.R.M.), Association pour la Recherche sur le Cancer (A.R.C.), Ligue Régionale contre le Cancer (Comité du Nord-Pas de Calais), Région Nord-Pas de Calais and Université de Lille II.

ABBREVIATIONS LIST

ACTR	=	Activator of thyroid and retinoic acid receptor
AF-1	=	Activation function 1 (ligand-independent)
AF-2	=	Activation function 2 (ligand-dependent)
AP1	=	Activating protein 1
atRA	=	All trans retinoic acid
bHLH	=	Basic helix-loop-helix
CARM-1	=	Coactivator associated arginine methyl transferase 1
CBP	=	CREB binding protein
DBD	=	DNA binding domain
DRIP	=	VDR interacting protein
ER	=	Estrogen receptor
FRK	=	Fos-regulating kinase
GR	=	Glucocorticoid receptor

GRIP	=	Glucocorticoid receptor interacting protein
HAT	=	Histone acetyl transferase
HRE	=	Hormone response element
JNK	=	Jun kinase
LBD	=	(Ligand binding domain or domain E
MAPK	=	Mitogen-activated protein kinases
NCoAs	=	Nuclear coactivator
NCoRs	=	Nuclear corepressor
NR	=	Nuclear receptor
PAS	=	<i>Period</i> , aryl hydrocarbon (dioxin) receptor, <i>single-minded</i>
PML	=	Promyelocytic leukemia
RARs	=	All trans retinoic acid receptors
RARE	=	Retinoic acid response element
RID	=	Receptor interaction domain
RXR	=	9-Cis retinoic acid receptors
SRC	=	Steroid receptor coactivator
TGF	=	Transforming growth factor
TIF	=	Transcriptional intermediary factor
TPA	=	12-O-tetradecanoylphorbol-13-acetate
TR	=	Thyroid hormone receptor
TRAP	=	TR interacting protein
VDR	=	Vitamin D receptor
pCAF	=	CRES-associated factor
pCIP	=	CREB-interacting protein
PPARs	=	Peroxisome proliferator activated receptors
VEGF	=	Vascular Endothelial Growth Factor

REFERENCES

- [1] Jensen, E. V., Jacobson, H. I. *Rec. Prog. Horm. Res.*, 1960, 18, 387-414.
- [2] Kenney, F. T., Wicks, W. D., Greenman, D. L. *J. Cell Physiol.*, 1965, 66, 27-36.
- [3] Kidson, C. *Biochem. Biophys. Res. Commun.*, 1965, 21, 283-289.

- [4] Dahmus, M. E.; Bonner, J. *Proc. Natl. Acad. Sci. U. S. A.*, 1965, **54**, 1370-1375.
- [5] Tomkins, G. M.; Martin, D. W. J. *Annu. Rev. Genet.*, 1970, **4**, 91-106.
- [6] Yamamoto, K. R.; Alberts, B. M. *Proc. Natl. Acad. Sci. U. S. A.*, 1972, **69**, 2105-2109.
- [7] O'Malley, B. W.; Rosenfeld, G. C.; Comstock, J. P.; Means, A. R. *Nat. New Biol.*, 1972, **240**, 45-48.
- [8] Norris, J. S.; Kohler, P. O. *Science*, 1976, **192**, 898-900.
- [9] Wrangé, O.; Gustafsson, J. A. J. *Biol. Chem.*, 1978, **253**, 856-865.
- [10] Pratt, W. B.; Silverstein, A. M.; Galigniana, M. D. *Cell Signal.*, 1999, **11**, 839-851.
- [11] Payvar, F.; Wrangé, Ö.; Carlstedt-Duke, J.; Okret, S.; Gustafsson, J. Å.; Yamamoto, K. R. *Proc. Natl. Acad. Sci. U. S. A.*, 1981, **78**, 6628-6632.
- [12] Payvar, F.; DeFranco, D. B.; Firestone, G. L.; Edgar, B. A.; Wrangé, Ö.; Okret, S.; Gustafsson, J. Å.; Yamamoto, K. R. *Cell*, 1983, **352**, 381-392.
- [13] Geisse, S.; Scheidereit, C.; Westphal, H. M.; Hynes, N. E.; Groner, B.; Beato, M. *EMBO J.*, 1982, **1**, 1613-1619.
- [14] Pfahl, M. *Cell*, 1982, **312**, 475-482.
- [15] Compton, J. G.; Schrader, W. T.; O'Malley, B. W. *Biochem. Biophys. Res. Commun.*, 1982, **105**, 96-104.
- [16] Pfahl, M.; McGinnis, D.; Hendricks, M.; Groner, B.; Hynes, N. E. *Science*, 1983, **222**, 1341-1343.
- [17] Zaret, K. S.; Yamamoto, K. R. *Cell*, 1984, **38**, 29-38.
- [18] Giguere, V. *Genet. Eng. N. Y.*, 1990, **12**, 183-200.
- [19] Thacher, S. M.; Vasudevan, J.; Chandraratna, R. A. S. *Curr. Pharm. Design.*, 2000, **6**, 25-58.
- [20] Lefebvre, B.; Mouchon, A.; Formstecher, P.; Lefebvre, P. *Biochemistry*, 1998, **37**, 9240-9249.
- [21] Montano, M. M.; Muller, V.; Trobaugh, A.; Katzenellenbogen, B. S. *Mol. Endocrinol.*, 1995, **9**, 814-825.
- [22] Dey, A.; Minucci, S.; Ozato, K. *Mol. Cell Biol.*, 1994, **14**, 8191-8201.
- [23] Blanco, J. C.; Dey, A.; Leid, M.; Minucci, S.; Park, B. K.; Jurutka, P. W.; Haussler, M. R.; Ozato, K. *Genes Cells*, 1996, **12**, 209-221.
- [24] Depoix, C.; Delmotte, M. H.; Formstecher, P.; Lefebvre, P. *J. Biol. Chem.*, 2001, **276**, 9452-9459.
- [25] Zechel, C.; Shen, X. Q.; Chambon, P.; Gronemeyer, H. *EMBO J.*, 1994, **13**, 1414-1424.
- [26] Zechel, C.; Shen, X. Q.; Chen, J. Y.; Chen, Z. P.; Chambon, P.; Gronemeyer, H. *EMBO J.*, 1994, **13**, 1425-1433.
- [27] Umesono, K.; Murakami, K. K.; Thompson, C. C.; Evans, R. M. *Cell*, 1993, **65**, 1255-1266.
- [28] Sanguedolce, M. V.; Leblanc, B. P.; Betz, J. L.; Stunnenberg, H. G. *EMBO J.*, 1997, **16**, 2861-2873.
- [29] Kurokawa, R.; Drenzo, J.; Boehm, M.; Sugarman, J.; Gloss, B.; Rosenfeld, M. G.; Heyman, R. A.; Glass, C. K. *Nature*, 1994, **371**, 528-531.
- [30] Kurokawa, R.; Soderstrom, M.; Horlein, A.; Halachmi, S.; Brown, M.; Rosenfeld, M. G.; Glass, C. K. *Nature*, 1995, **377**, 451-454.
- [31] Bourguet, W.; Ruff, M.; Chambon, P.; Gronemeyer, H.; Moras, D. *Nature*, 1995, **375**, 377-382.
- [32] Renaud, J. P.; Rochel, N.; Ruff, M.; Vivat, V.; Chambon, P.; Gronemeyer, H.; Moras, D. *Nature*, 1995, **378**, 681-689.
- [33] Egea, P. F.; Mitschler, A.; Rochel, N.; Ruff, M.; Chambon, P.; Moras, D. *EMBO J.*, 2000, **19**, 2592-2601.
- [34] Klaholz, B. P.; Renaud, J. P.; Mitschler, A.; Zusi, C.; Chambon, P.; Gronemeyer, H.; Moras, D. *Nat. Struct. Biol.*, 1998, **53**, 199-202.
- [35] Bourguet, W.; Vivat, V.; Wurtz, J. M.; Chambon, P.; Gronemeyer, H.; Moras, D. *Mol. Cell*, 2000, **5**, 289-298.
- [36] Ostrowski, J.; Roalsvig, T.; Hammer, L.; Marinier, A.; Starrett, J. E.; Yuo, K. L.; Reczek, P. R. *J. Biol. Chem.*, 1998, **273**, 3490-3495.
- [37] Scafanas, A.; Wolfgang, C. L.; Gabriel, J. L.; Soprano, K. J.; Soprano, D. R. *J. Biol. Chem.*, 1997, **272**, 11244-11249.
- [38] Klaholz, B. P.; Mitschler, A.; Moras, D. *J. Mol. Biol.*, 2000, **302**, 155-170.
- [39] Benkoussa, M.; Nomine, B.; Mouchon, A.; Lefebvre, B.; Bernardon, J. M.; Formstecher, P.; Lefebvre, P. *Recept. Signal. Transduct.*, 1997, **74**, 257-267.
- [40] Danielian, P. S.; White, R.; Lees, J. A.; Parker, M. G. *EMBO J.*, 1992, **11**, 1025-1033.
- [41] Mouchon, A.; Delmotte, M.-H.; Formstecher, P.; Lefebvre, P. *Mol. Cell Biol.*, 1999, **19**, 3073-3085.
- [42] Lefebvre, B.; Mouchon, A.; Formstecher, P.; Lefebvre, P. *Mol. Cell Endocrinol.*, 1998, **139**, 161-169.
- [43] Rachez, C.; Sautiere, P.; Formstecher, P.; Lefebvre, P. *J. Biol. Chem.*, 1996, **271**, 17996-18006.
- [44] Meyer, M. E.; Gronemeyer, H.; Turcotte, B.; Bocquel, M. T.; Tasset, D.; Chambon, P. *Cell*, 1989, **57**, 433-442.
- [45] Lee, J. W.; Choi, H. S.; Gyuris, J.; Brent, R.; Moore, D. D. *Mol. Endocrinol.*, 1995, **9**, 243-254.
- [46] Lee, J. W.; Ryan, F.; Swaffield, J. C.; Johnston, S. A.; Moore, D. D. *Nature*, 1995, **374**, 91-94.
- [47] Seol, W. G.; Choi, H. S.; Moore, D. D. *Mol. Endocrinol.*, 1995, **9**, 72-85.
- [48] Rachez, C.; Suldán, Z.; Ward, J.; Chang, C. P. B.; Burakov, D.; Erdjument-Bromage, H.; Tempst, P.; Freedman, L. P. *Genes & Develop.*, 1998, **12**, 1787-1800.

- [49] Onate, S. A., Tsai, S. Y., Tsai, M. J.; O'Malley, B. W. *Science*, 1995, 270, 1354-1357.
- [50] Leo, C.; Chen, J. D. *Gene*, 2000, 245, 1-11.
- [51] Chen, H. W., Lin, R. J., Schiltz, R. L., Chakravarti, D., Nash, A., Nagy, L., Privalsky, M. L., Nakatani, Y.; Evans, R. M. *Cell*, 1997, 90, 569-580.
- [52] Spencer, T. E., Jenster, G., Burcin, M. M., Allis, C. D., Zhou, J. X., Mizzen, C. A., McKenna, N. J., Onate, S. A., Tsai, S. Y., Tsai, M. J.; O'Malley, B. W. *Nature*, 1997, 389, 194-198.
- [53] Chen, H. W., Lin, R. J., Xie, W., Wilpitz, D.; Evans, R. M. *Cell*, 1999, 98, 675-686.
- [54] Rachez, C.; Freedman, L. P. *Gene*, 2000, 246, 9-21.
- [55] Westin, S., Rosenfeld, M. G.; Glass, C. K. *Advances in Pharmacology*, 2000, 47, 89-112.
- [56] Sauve, F., McBroom, L. D., Gallant, J., Moraitis, A. N., Labrie, F.; Giguere, V. *Mol. Cell Biol.*, 2001, 21, 343-353.
- [57] Voegel, J. J., Heine, M. J. S., Tini, M., Vivat, V., Chambon, P.; Gronemeyer, H. *EMBO J.*, 1998, 17, 507-519.
- [58] McInerney, E. M., Rose, D. W., Flynn, S. E., Westin, S., Mullen, T. M., Kronos, A., Inostroza, J., Torchia, J., Nolte, R. T., Assamunt, N., Milburn, M. V., Glass, C. K.; Rosenfeld, M. G. *Genes & Develop.*, 1998, 12, 3357-3368.
- [59] Eckner, R. *Biol. Chem.*, 1996, 377, 685-688.
- [60] Hanstein, B., Eckner, R., Drenzo, J., Halachmi, S., Liu, H., Searcy, B., Kurokawa, R.; Brown, M. *Proc. Natl. Acad. Sci. U. S. A.*, 1996, 93, 11540-11545.
- [61] Chakravarti, D., LaMorte, V. J., Nelson, M. C., Nakajima, T., Schulman, I. G., Juguilon, H., Montminy, M.; Evans, R. M. *Nature*, 1996, 383, 99-103.
- [62] Kamei, Y., Xu, L., Heinzel, T., Torchia, J., Kurokawa, R., Gloss, B., Lin, S. C., Heyman, R. A., Rose, D. W., Glass, C. K.; Rosenfeld, M. G. *Cell*, 1996, 85, 403-414.
- [63] Yao, T. P., Ku, G., Zhou, N., Scully, R.; Livingston, D. M. *Proc. Natl. Acad. Sci. U. S. A.*, 1996, 93, 10626-10631.
- [64] Westin, S., Kurokawa, R., Nolte, R. T., Wisely, G. B., McInerney, E. M., Rose, D. W., Milburn, M. V., Rosenfeld, M. G.; Glass, C. K. *Nature*, 1998, 395, 199-202.
- [65] Ogryzko, V. V., Schiltz, R. L., Russanova, V., Howard, B. H.; Nakatani, Y. *Cell*, 1996, 87, 953-957.
- [66] Korzus, E., Torchia, J., Rose, D. W., Xu, L., Kurokawa, R., McInerney, E. M., Mullen, T. M., Glass, C. K.; Rosenfeld, M. G. *Science*, 1998, 279, 703-707.
- [67] Xu, L., Glass, C. K.; Rosenfeld, M. G. *Curr. Opin. Genet. Dev.*, 1999, 9, 140-147.
- [68] Fondell, J. D., Ge, H.; Roeder, R. G. *Proc. Natl. Acad. Sci. U. S. A.*, 1996, 93, 8329-8333.
- [69] Ito, M., Yuan, C. X., Malik, S., Gu, W., Fondell, J. D., Yamamura, S., Fu, Z. Y., Zhang, X. L., Qin, J.; Roeder, R. G. *Mol. Cell*, 1999, 3, 361-370.
- [70] Rachez, C., Lemon, B. D., Suldan, Z., Bromleigh, V., Gamble, M., Naar, A. M., Erdjument-Bromage, H., Tempst, P.; Freedman, L. P. *Nature*, 1999, 398, 824-828.
- [71] Naar, A. M., Beaurang, P. A., Robinson, K. M., Oliner, J. D., Avizonis, D., Scheek, S., Zwicker, J., Kadonaga, J. T.; Tjian, R. *Genes & Develop.*, 1998, 12, 3020-3031.
- [72] Naar, A. M., Beaurang, P. A.; Zhou, S., Abraham, S., Solomon, W.; Tjian, R. *Nature*, 1999, 398, 828-832.
- [73] Boyer, T. G., Martin, M. E., Lees, E., Ricciardi, R. P.; Berk, A. J. *Nature*, 1999, 399, 276-279.
- [74] Sun, X., Zhang, Y., Cho, H., Rickert, P., Lees, E., Lane, W.; Reinberg, D. *Mol. Cell*, 1998, 2, 213-222.
- [75] Gu, W., Malik, S., Ito, M., Yuan, C. X., Fondell, J. D., Zhang, X. L., Martinez, E., Qin, J.; Roeder, R. G. *Mol. Cell*, 1999, 3, 97-108.
- [76] Lorch, Y., Beve, J., Gustafsson, C. M., Myers, L. C.; Kornberg, R. D. *Mol. Cell*, 2000, 6, 197-201.
- [77] Robyr, D., Wolffe, A. P.; Wahli, W. *Mol. Endocrinol.*, 2000, 14, 329-347.
- [78] Blanco, J. C. G., Minucci, S., Lu, J. M., Yang, X. J., Walker, K. K., Chen, H. W., Evans, R. M., Nakatani, Y.; Ozato, K. *Genes & Develop.*, 1998, 12, 1638-1651.
- [79] Chen, D. G., Huang, S. M.; Stallcup, M. R. *J. Biol. Chem.*, 2000, 275, 40810-40816.
- [80] Chen, D., Ma, H., Hong, H., Koh, S. S., Huang, S. M., Schurter, B. T., Aswad, D. W.; Stallcup, M. R. *Science*, 1999, 284, 2174-2177.
- [81] Fraser, R. A., Heard, D. J., Adam, S., Lavigne, A. C., Ledouarin, B., Tora, L., Losson, R., Rochette-Egly, C.; Chambon, P. *J. Biol. Chem.*, 1998, 273, 16199-16204.
- [82] Nolte, R. T., Wisely, G. B., Westin, S., Cobb, J. E., Lambert, M. H., Kurokawa, R., Rosenfeld, M. G., Willson, T. M., Glass, C. K.; Milburn, M. V. *Nature*, 1998, 395, 137-143.
- [83] Gampe, R. T., Montana, V. G., Lambert, M. H., Miller, A. B., Bledsoe, R. K., Milburn, M. V., Kliewer, S. A., Willson, T. M.; Xu, H. E. *Mol. Cell*, 2000, 5, 545-555.
- [84] Kalkhoven, E., Valentine, J. E., Heery, D. M.; Parker, M. G. *EMBO J.*, 1998, 17, 232-243.
- [85] Leers, J., Treuter, E.; Gustafsson, J. A. *Mol. Cell Biol.*, 1998, 18, 6001-6013.
- [86] Ding, X. F., Anderson, C. M., Ma, H., Hong, H., Uht, R. M., Kushner, P. J.; Stallcup, M. R. *Mol. Endocrinol.*, 1998, 12, 302-313.
- [87] Darimont, B. D., Wagner, R. L., Apriletti, J. W., Stallcup, M. R., Kushner, P. J., Baxter, J. D., Fletterick, R. J.; Yamamoto, K. R. *Genes & Develop.*, 1998, 12, 3343-3356.

- [88] Chang, C., Norris, J. D., Gron, H., Paige, L. A., Hamilton, P. T., Kenan, D. J., Fowlkes, D.; McDonnell, D. P. *Mol. Cell. Biol.*, 2001, 19, 8226-8239.
- [89] Angel, P., Imagawa, M., Chiu, R., Stein, B., Jonat, C.; Karin, M. *Cell*, 1987, 49, 729-739.
- [90] Karin, M., Liu, Z.; Zandi, E. *Curr. Opin. Cell Biol.*, 1997, 9, 240-246.
- [91] Cook, S. J., Aziz, N.; McMahon, M. *Mol. Cell Biol.*, 1999, 19, 330-341.
- [92] Karin, M. *Philos. Trans. R. Soc. Lond. Biol.*, 1996, 351, 127-134.
- [93] Bannister, A. J., Oehler, T., Wilhelm, D., Angel, P.; Kouzarides, T. *Oncogene*, 1995, 11, 2509-2514.
- [94] Deng, T.; Karin, M. *Nature*, 1994, 371, 171-175.
- [95] Young, M. R., Li, J. J., Rincon, M., Flavell, R. A., Sathyanarayana, B. K., Hunziker, R.; Colburn, N. *Proc. Natl. Acad. Sci. U. S. A.*, 1999, 96, 9827-9832.
- [96] Schule, R., Rangarajan, P., Yang, N., Kliewer, S., Ransone, L. J., Bolado, J., Verma, I. M.; Evans, R. M. *Proc. Natl. Acad. Sci. U. S. A.*, 1991, 88, 6092-6096.
- [97] Caelles, C., GonzalezSancho, J. M.; Munoz, A. *Genes & Develop.*, 1997, 11, 3351-3364.
- [98] Lee, H. Y., Sueoka, N., Hong, W. K., Mangelsdorf, D. J., Claret, F. X.; Kurie, J. M. *Mol. Cell Biol.*, 1999, 19, 1973-1980.
- [99] Fanjul, A., Dawson, M. I., Hobbs, P. D., Jong, L., Cameron, J. F., Harlev, E., Graupner, G.; Lu, X. P.; Pfahl, M. *Nature*, 1994, 372, 107-111.
- [100] Nagpal, S., Athanikar, J.; Chandraratna, R. A. S. *J. Biol. Chem.*, 1995, 270, 923-927.
- [101] Lippman, S. M.; Lotan, R. *J. Nutr.*, 2000, 130, 479S-482S.
- [102] Bayaa, M., Booth, R. A., Sheng, Y. L.; Liu, X. J. *Proc. Nat. Acad. Sci. U. S. A.*, 2000, 97, 12607-12612.
- [103] Chomienne, C., Fenaux, P.; Degos, L. *FASEB J.*, 1996, 10, 1025-1030.
- [104] Nagpal, S.; Chandraratna, R. A. S. *Curr. Pharm. Design.*, 2000, 6, 919-931.
- [105] Saiag, P., Pavlovic, M., Clerici, T., Feauveau, V., Nicolas, J. C., Emilie, D.; Chastang, C. *AIDS*, 1998, 12, 2169-2176.
- [106] Nagpal, S., Cai, J., Zheng, T., Patel, S., Masood, R., Lin, G. Y., Friant, S., Johnson, A., Smith, D. L., Chandraratna, R. A. S.; Gill, P. S. *Mol. Cell Biol.*, 1997, 17, 4159-4168.
- [107] Corbeil, J., Rapaport, E., Richman, D. D.; Looney, D. J. *J. Clin. Invest.*, 1994, 93, 1981-1986.
- [108] Wang, Z. Y., Chen, Z., Huang, W., Li, X. S., Lu, J. X., Huang, L. A., Zhang, F. Q., Gu, L. J., Ouyang, R. R., Chen, S. J.; Sun, G. L. *Blood Cells*, 1993, 19, 633-641.
- [109] Standeven, A. M., Teng, M.; Chandraratna, R. A. *Toxicol. Lett.*, 1997, 92, 231-240.
- [110] Huang, M. E., Ye, Y. C., Chen, S. R., Chai, J. R., Lu, J. X., Zhao, L., Gu, L. J.; Wang, Z. Y. *Blood*, 1988, 72, 567-572.
- [111] Chen, Y., Takeshita, A., Ozaki, K., Kitano, S.; Hanazawa, S. *J. Biol. Chem.*, 1996, 271, 31602-31606.
- [112] Kitamura, K., Kiyoi, H., Yoshida, H., Tobita, T., Takeshita, A., Ohno, R.; Naoe, T. *Cancer Chemother. Pharmacol.*, 1997, 40, S36-S41.
- [113] Huang, C. S., Ma, W. Y., Dawson, M. I., Rincon, M., Flavell, R. A.; Dong, Z. G. *Proc. Natl. Acad. Sci. U. S. A.*, 1997, 94, 5826-5830.
- [114] Vega-Diaz, B., Lenoir, M. C., Ladoux, A., Frelin, C., Démarchez, M.; Michel, S. *J. Biol. Chem.*, 2000, 275, 642-650.
- [115] Triozzi, P. L., Walker, M. J., Pellegrini, A. E.; Dayton, M. A. *Cancer Invest.*, 1996, 14, 293-298.
- [116] Oikawa, T., Hirotsu, K., Nakamura, O., Shudo, K., Hiragun, A.; Iwaguchi, T. *Cancer Lett.*, 1989, 48, 157-162.
- [117] Kizaki, M., Dawson, M. I., Heyman, R., Elstner, E., Morosetti, R., Pakkala, S., Chen, D. L., Ueno, H., Chao, W. R., Morikawa, M., Ikeda, Y., Heber, D., Pfahl, M.; Koeffler, H. P. *Blood*, 1996, 87, 1977-1984.
- [118] Evans, T. R.; Kaye, S. B. *Br. J. Cancer*, 1999, 80, 1-8.
- [119] McDonnell, D. P. *Trends Endocrinol. Metab.*, 1999, 10, 301-311.
- [120] Delmotte, M. H., Tahayato, A., Formstecher, P.; Lefebvre, P. *J. Biol. Chem.*, 1999, 274, 38225-38231.